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Synthesis of ^{14}C-Lanosterol and ^{14}C-Desmosterol [1]

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SUMMARY

The synthesis of lanosterol [*I*a] *and desmosterol* [*II*a] *labeled with* ^{14}*C in the 25 position by a Wittig reaction between triphenyl-2*^{14}*C isopropylidene-phosphorane* [*IV*b] *and the corresponding aldehydes are described. The specific activities obtained are, respectively, 0.539 mC/mM and 0.752 mC/mM and the purities of the compounds, respectively, 96 and 99 %.*

INTRODUCTION

The availability of labeled lanosterol [I*a*] and desmosterol [II*a*] can be useful to research on steroid biosynthesis and metabolism. During some research along those lines we have therefore synthesized [I*a*] and [II*a*] labeled with ^{14}C in the 25 position.

The synthesis of labeled desmosterol has been carried out according to the scheme drawn by U. H. M. FAGERLUND and D. R. IDLER [1] for the unlabeled compound, but we modified the experimental procedure in order to obtain higher yields based on the 2^{14}C-isopropyl halide (Scheme 1).

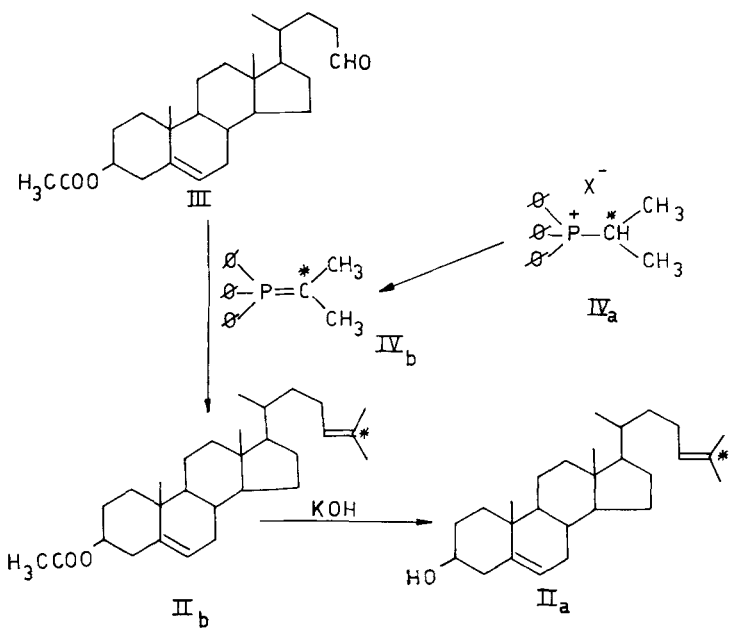
In the procedure of Fagerlund desmosterol was synthesized by a Wittig reaction between 3ß-acetoxy-5-cholen-24-al [III] and triphenyl-isopropyliden-phosphorane [IV*b*]; this latter was obtained by treatment of the bromide (IV*a*; X = Br) with butyl-lithium.

Following this method the yields were 25 % based on [III] but only 3.7 % based on isopropyl bromide.

We found that using triphenyl-isopropyl-phosphonium iodide and a solution of methyl-sulfinyl-carbanion in dimethyl-sulfoxide [2] as a base, the

[1] Work carried out in the field of a Euratom contract.

yields in the synthesis of [IV*b*] were considerably improved; an equimolecular quantity of (IV*a*; X = I) was first added to this solution and then an equimolecular quantity of [III]. The average yields in [II*b*] for a series of runs with unlabeled compounds were 24.4 % based on [III] and 17.1 % based on isopropyl iodide.

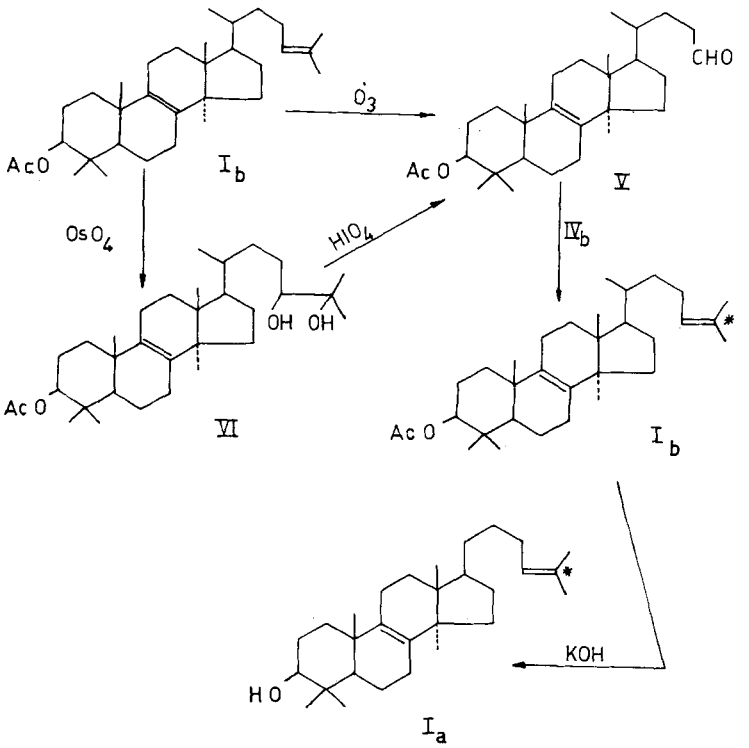


SCHEME I.

The synthesis of 25 ^{14}C-lanosterol was carried out in a similar manner starting from 3ß-acetoxy-25, 26, 27-trisnor-8-lanosten-24-al [V], obtained by oxidative degradation of lanosterol (Scheme 2). The isolation of the latter from the mixture of triterpene alcohols of wool fat (« isocholesterol ») by the procedure of D. J. JOHNSON, F. GAUTSCHI and K. BLOCH [3] gives constantly a material which is a mixture of lanosterol and 24-dihydrolanosterol in considerable amounts, as evidenced by thin layer chromatography on silica gel-$AgNO_3$.

We obtained, however, a very pure product by column chromatography of isocholesteryl acetate on $AgNO_3$—silica gel—celite. The aldehyde [V] was prepared by periodic acid oxidation of 3ß-acetoxy-8-lanostene-24, 25-diol [VI] obtained by the procedure of H. WIELAND and W. BENEND [4], or, with comparable yields but more quickly and economically, by selective ozonolysis of lanosteryl acetate [I*b*] [5].

If the aldehyde is not requested at very high purity, the ozonolysis can be carried out directly on isocholesteryl acetate. Ozonolysis of [*Ib*] had previously been effected by T. G. HALSALL and R. HODGES [6], but they did not isolate the aldehyde [V].



SCHEME II.

EXPERIMENTAL PART

3β-acetoxy-lanosterol from « isocholesteryl acetate ».

A suspension of 150 g of Mallinckrodt silica gel and 150 g of celite in 800 ml of water containing 300 g of silver nitrate was heated in a water bath for 1 h with efficient stirring; after removal of the water under vacuum, the dried mixture was activated by heating 16 h at 120° C and was finely pulverized. 500 g of adsorbing mixture were washed with 600 ml of boiling petroleum ether, then with 600 ml of dry benzene and lastly with 1 l of dry petroleum ether. 6 g of isocholesteryl acetate were chromatographed on this mixture. The results of the chromatography are reported in Table I.

TABLE I.

Fractions	Eluent	Substance	Weight
1-15	Petroleum ether-benzene 97/3 (v/v)	Dihydrolanosteryl acetate	1.611 g
16-28	Petroleum ether-benzene 90/10 (v/v)	Dihydrolanosteryl acetate + lanosteryl acetate	1.280 g
29-32	Petroleum ether-benzene 75/25 (v/v)	Lanosteryl acetate	2.200 g
33-40	Benzene	More polar products	1.850 g

The pure lanosteryl acetate (thin layer chromatography on $AgNO_3$-silicagel) was crystallized from methanol; m.p. 129°-130° C.

Anal. found % : C 82.17; H 10.98;

Calcd. for $C_{32}H_{52}O_2$: C 81.99; H 11.18.

3ß acetoxy-25, 26, 27 trisnor-8 lanosten-24 al [*V*]

a) By periodic acid oxidation of [VI]

To a solution of 0.8 g of [VI] [4] in 46 ml of ethanol were added 9 ml of pyridine and 3.5 ml of HIO_4 1 M. After 15 h at room temperature the mixture was poured in ice water and extracted with ether; the ether layer was washed with sodium hydrogen carbonate solution, dilute hydrochloric acid and water, then dried and evaporated to dryness. The crude product (0.704 g) was repeatedly crystallized from acetic acid; m.p. 139°-142° C.

Anal. found % : C 78.38; H 10.22;

Calcd. for $C_{29}H_{46}O_3$: C 78.68; H 10.47.

The semicarbazone, prepared in the standard manner, was crystallized from methanol-chloroform; m.p. 215°-218° C.

Anal. found % : C 72.14; H 9.62; N 8.25;

Calcd. for $C_{30}H_{49}N_3O_3$: C 72.10; H 9.88; N 8.41.

b) By ozonolysis of lanosteryl acetate.

1.534 g of [I*b*] were dissolved in 300 ml of chloroform and ozonized at —20° C for 14 min. with a stream of air containing 1.2 g/h of ozone. The solution was treated, with agitation, by a saturated solution of $FeSO_4$, testing for the disappearance of ozonides with iodine-starch paper.

The organic layer was washed with water, dried and evaporated. The solid residue was chromatographed on alumina III. The petroleum ether

eluted 0.53 g of lanosteryl acetate, and then the petroleum ether-benzene mixture 3 : 1 eluted 0.407 g of the aldehyde [V]. The recovered lanosteryl acetate was re-ozonized as described above, giving 0.120 g of [V]. After recrystallization from acetic acid the product has the same melting point as that in *a*).

c) By ozonolysis of « isocholesteryl » acetate.

A solution of 0.43 g of « isocholesteryl » acetate in 40 ml of chloroform was ozonized for 4 min. and 15 sec. as in *b*). After reduction of the ozonide with $FeSO_4$, the crude product was chromatographed on alumina III, giving 90 mg of [V] identical with the product prepared as previously described.

*Triphenyl-2^{14}C isopropyl-phosphonium iodide (IV*a; *X = I).*

0.83 g of triphenyl-phosphine and 0.49 g of 2^{14}C-isopropyl iodide (activity 0.695 mC/mmole) were heated under vacuum for 24 h at 150° C in a sealed tube. The product was crystallized three times in a nitrogen atmosphere from absolute ethanol-ether, filtered, dried under vacuum and kept in the dark under nitrogen atmosphere. The yields were 0.79 g; m.p. 188° C. In order to find the best experimental conditions, a series of runs had been carried out with unlabeled compounds; the results are reported in Table II.

TABLE II.

		T° C	*t*	Yields	m.p.
g 0.576	g 0.34	150	24 h	g 0.623	188
g 0.576	g 0.34	100	24 h	g 0.574	188
g 0.576	g 0.34	80	24 h	g 0.368	188
g 0.576	g 0.34	150 × 12 h; then 100 × 12 h	24 h	g 0.485	188

Desmosterol 25^{14}C [*II*b].

In a 25 ml two-necked flask, 44 mg of sodium hydride (50 % in oil) were washed repeatedly, with efficient stirring, with ether dried on calcium hydride, the ether being decanted after each washing. The hydride was dried under vacuum. Then, under a nitrogen atmosphere, 0.5 ml of dimethyl-sulfoxide dried on calcium hydride, were added with a syringe. The mixture was heated

at 70-80° C for 45 min. with stirring. During this time hydrogen evolved and the solution became green-grey. To the cooled solution were added, with a syringe, 0.395 g of (IV*a*; X = I) dissolved in 1 ml of dimethyl-sulfoxide (the colour became deep red). After 10 min. at room temperature, 0.4 g of [III] [1] (purified by chromatography on SiO_2 Merck 0.2-0.5 mm, eluent petroleum ether-benzene 1/1 *v*/*v*), dissolved in 5 ml of dry tetrahydrofuran, were added. The colour disappeared immediately; after 24 h at 50° C under nitrogen the solution was cooled, poured in ice water and extracted with ether. The ether layer was washed with water, dried and evaporated to dryness. The solid residue (0.5 g) was directly acetylated with 1 ml of acetic anhydride and 10 ml of pyridine at room temperature overnight. The crude product obtained (0.574 g) was chromatographed on 100 g of SiO_2 Merck (0.2-0.5 mm). Elution with petroleum ether-benzene 1/1 (*v*/*v*) gave 0.104 g of [II*b*], which was hydrolized by refluxing for 2 h with 22 ml of KOH 0.08 N in methanol. The cooled mixture was poured in ice water and extracted with ether. The ethereal layer washed, dried and evaporated, gave 0.1 g of desmosterol $25^{14}C$ [II*a*]. The purity of the product was higher than 99 % (Fig. 1).

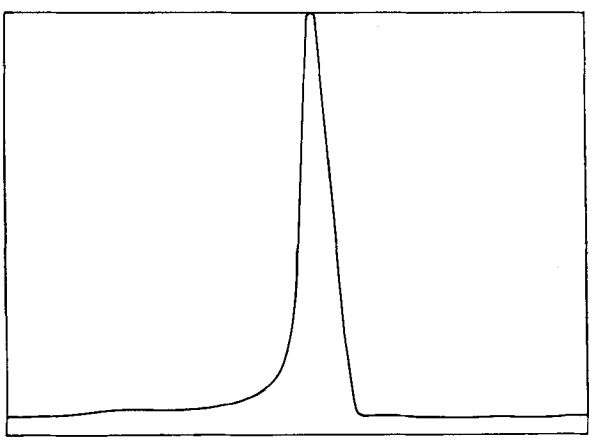


FIG. 1. — Gas-chromatogram of ^{14}C-Desmosterol (II*a*)

Lanosterol $25^{14}C$ [*I*a].

44 mg of sodium hydride, 0.395 g of [IV*b*] and 0.41 g of [V] were treated as previously described for the synthesis of [II*a*]. The crude product (0.62 g) was acetylated and chromatographed on 15 g of alumina III. Elution with petroleum ether gave 70 mg of 3ß-acetoxy-lanosterol [I*b*]. By refluxing with 20 ml of KOH 0.08 N in methanol for 3 h, the latter product gave 62 mg of lanosterol $25^{14}C$ [I*a*], about 96 % pure (Fig. 2).

The gas-phase-chromatographic identification of [I*a*] and [II*a*] was carried out with a Carlo Erba Fractovap model C with flame ionization detector. Column : PhSi 191-43 on Gas Chrom P 100-120 mesh, inactivated according to HORNING and coll. [7].

Temperature : column 223° C, injector 260° C, Gas carrier : N_2.

The identification of [I*a*] and [II*a*] was effected by their retention times being referred to cholestane and by comparison with authentic specimens.

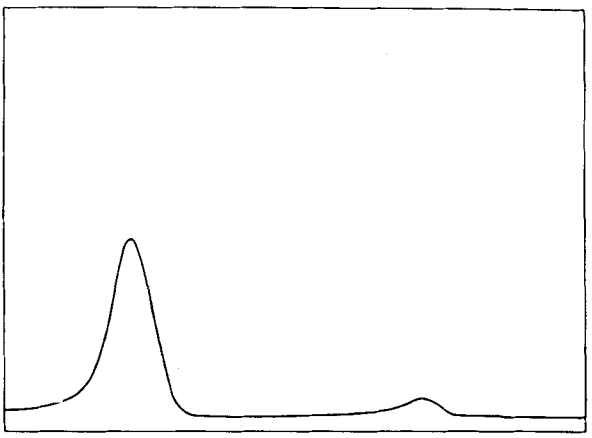


FIG. 2. — Gas-chromatogram of C^{14}-Lanosterol (I*a*).

Determination of specific radioactivity.

Samples of [I*a*] and [II*a*] were dissolved in petroleum ether and added to a solution of 65 mg of PPO and 1.3 mg of dimethyl POPOP. The radioactivity was tested with a Packard Liquid Scintillator Spectrometer TRI-CARB-3000. The standard had an efficiency of 79.59 %. The activity of [II*a*] was 0.752 mC/mmole and that of [I*a*] was 0.539 mC/mmole.

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   The physical constants, however, of the product described by these authors do not agree with those of the aldehyde obtained by our procedure.
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